

Remarks

Reconsideration of this Application is respectfully requested.

Page 1 has been amended to include a cross-reference to the related application.

The paragraph bridging pages 8-9 has been amended to include sequence ID numbers and to correct minor typographical errors. In addition, paper and computer readable copies of the Sequence Listing are submitted herewith.

Upon entry of the foregoing amendment, claims 1, 2, 4, 5, 9-25, 28-41, 43-56, and 58-70 are pending in the application. Claims 3, 6, 7, 8, 26, 27, 42 and 57 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein.

Claims 1, 34-41, 43, 44, 47, 53 and 54 are sought to be amended. Support for the amendments to claims 1, 53 and 54 may be found throughout the application and, in particular, on page 11, line 12, and claim 3. The amendments to claims 34-41, 43, 44 and 47 are to clarify the language and have support as well throughout the application. Support for new claims 59-61 and 67-69 may be found on page 11, lines 10-13. Support for new claims 63-66 may be found on page 18, lines 15-19. Support for new claim 70 may be found throughout the application and, in particular, on page 4, lines 7-9 and 18-20; page 7, line 16, through page 10, line 17, and claims 41-44 and 47-52. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Sequence Listing

On page 2 of the Office Action, the Examiner states that the application does not comply with the sequence listing rules.

Applicants submit herewith a paper and computer readable copy of a sequence listing comprising the sequences set forth on page 9 of the application. The paper copy of the sequence listing and the computer readable copy of the sequence listing are the same. Applicants have also amended the paragraph bridging pages 8-9 to include appropriate sequence listing numbers. Withdrawal of the objection is respectfully requested.

Rejections under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected claims 33-34 and 47-50 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim what Applicants regard as their invention. Applicants respectfully traverse this rejection.

Claim 42 has been canceled thus rendering moot this portion of the rejection.

Applicants submit that the claims have been amended to delete reference to "biological" and "molecular" probes, thus obviating this aspect of the rejection. In addition, claims 38 and 39 have been amended to delete recitation of "sequences thereof," thus obviating this aspect of the rejection.

Withdrawal of the rejection to the claims under 35 U.S.C. § 112, second paragraph, is respectfully requested.

Rejections under 35 U.S.C. § 112, First Paragraph

The Examiner has rejected claims 24-27 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicants respectfully traverse this rejection.

Claims 26 and 27 have been cancelled thus rendering moot this basis for rejection.

According to the Examiner:

the instant application teaches only the selection of probes which can be distinguished from each one another by the use of spectral filters. The specification does not teach a special spectral filter, a digital imaging system or data analysis system, nor does it teach specific probes which have maximum emission peaks at 772 nm and 795 nm. One of skill in the art would not be able to make or use said probe without being forced into undue experimentation to make fluorescent probes having the emission spectra as claimed.

Office Action at page 4, lines 4-9.

Applicants respectfully disagree with the Examiner's contentions. The Examiner has the burden to establish a reasonable basis to question the enablement provided for the claimed invention. A specification disclosure which contains a teaching of the manner

and process of making and using an invention must be taken as being in compliance with the enablement requirement of 35 U.S.C. §112, first paragraph, unless there is a reason to doubt the truth of the statements contained therein. See M.P.E.P. §2164.04. Applicants submit that the Examiner has not met this burden.

An applicant is not limited to the confines of the specification to provide the necessary information to enable the invention. *In re Howarth*, 654 F.2d 103, 105-6, 210 U.S.P.Q. 689, 692 (C.C.P.A. 1981). An applicant need not supply information that is well known in the art. *Howarth*, 654 F.2d at 105-6, 210 U.S.P.Q. at 692; *see also In re Brebner*, 455 F.2d 1402, 173 U.S.P.Q. 169 (C.C.P.A. 1972) (finding a disclosure enabling because the procedure for making the starting material, although not disclosed, would have been known to one of ordinary skill in the art as evidenced by a Canadian patent). "That which is common and well known is as if it were written out in the patent and delineated in the drawings." *Howarth*, 654 F.2d at 106, 210 U.S.P.Q. at 692 (quoting *Webster Loom Co. v. Higgins et al.*, 105 U.S. (15 Otto.) 580, 586 (1881)). Moreover, one of ordinary skill in the art is deemed to know not only what is considered well known in the art but also where to search for any needed materials. *Id.*

As acknowledged by the Examiner, Roederer *et al.* teach the probe Cy7 that has an emission maxima of 778 nm (see also Waggoner *et al.*, at page 497, cited by Applicants as document AT15) ^{Or} which is "about 772." Also acknowledged by the Examiner is the known "complex digital imaging and data analysis of probes which have been labeled by mixtures of known fluorophores to circumvent this problem of spectral overlap. . ." Office Action at page 4, lines 1-2. In view of the known spectral filters, digital imaging systems, data analysis systems and probes, Applicants submit that the

invention of claims 24 and 25 is fully enabled. The state of the art at the time of Applicants' invention would not have required undue experimentation to practice the claimed invention.

Withdrawal of the rejection under 35 U.S.C. § 112, first paragraph, is respectfully requested.

Rejections under 35 U.S.C. § 102

The Examiner has rejected claims 1, 6, 9-13, 14-19, 22, 23, 28-30, 33-37, 40-42, 44-46, 51, 52 and 54 under 35 U.S.C. § 102(b) as being anticipated by Dow *et al.*, *Cytometry* 25:71-81 (1996). Applicants respectfully traverse this rejection.

Claims 6 and 42 have been canceled thus rendering moot this portion of the rejection.

To anticipate a claim, a reference must teach every element of that claim. Dow *et al.* teach fluorescence imaging of tissue sections. Dow *et al.* do not teach a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. Therefore, Dow *et al.* do not anticipate any one of claims 1, 9-13, 14-19, 22, 23, 28-30, 33-37, 40-41, 44-46, 51, 52 and 54. Withdrawal of the rejection is respectfully requested.

The Examiner has rejected claims 1, 3, 5, 6, 11, 12, 13-19, 22, 23, 28-33, 37, 38, 40-42, 45 and 54 under 35 U.S.C. § 102(b) as being anticipated by Ried *et al.*, *Proc. Natl. Acad. Sci. USA* 89:1388-1392 (1992). Applicants respectfully traverse this rejection.

Claims 3, 6 and 42 have been canceled thus rendering moot this portion of the rejection.

Ried *et al.* teach the visualization of human metaphase chromosomes with labeled DNA probes. Ried *et al.* do not teach a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. Therefore, Ried *et al.* do not anticipate any one of claims 1, 5, 11, 12, 13-19, 22, 23, 28-33, 37, 38, 40-41, 45 and 54. Withdrawal of the rejection is respectfully requested.

The Examiner has rejected claims 1, 3-5, 7-9, 11-13, 16, 17, 20, 21, 28, 29, 33-36, 40, 41, 43-46 and 53-57 under 35 U.S.C. § 102(b) as being anticipated by Gross *et al.*, *Proc. Natl. Acad. Sci. USA* 92:537-541 (1995). Applicants respectfully traverse this rejection.

Claims 3, 7, 8 and 57 have been canceled thus rendering moot this portion of the rejection.

Gross *et al.* disclose a *model* study showing detection of breast cancer cells seeded into a suspension of isolated peripheral blood mononuclear cells. See page 538, col. 1, first, second and fourth full paragraphs. Gross *et al.* do not teach a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. Thus, Gross *et al.* do not anticipate claims 1, 4, 5, 9, 11-13, 16, 17, 20, 21, 28, 29, 33-36, 40, 41, 43-46 and 53-56. Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1, 2-5, 7-13, 16, 17, 20, 21, 28, 29, 33-36, 40, 41, 43-46 and 54 under 35 U.S.C. § 103(a) as being unpatentable over Gross *et al.* in view of "what is well known in the art as exemplified by Freshney (The culture of animal Cells, 3rd edition, 1994, pp. 185-189) and Ausubel et al (Short Protocols in Molecular Biology 2nd edition, 1992, pp. 14.9-14.11)." Page 7, lines 5-8. Applicants respectfully traverse this rejection.

Claims 3, 7 and 8 have been canceled thus rendering moot this portion of the rejection.

According to the Examiner:

The isolation and fixing of cells is not taught specifically by Gross et al. The fixing of cells in preparation for in situ hybridization is taught by Ausubel et al. The isolation of cells by density gradient is taught by Freshney. It would have been *prima facia* [sic] obvious to one of ordinary skill in the art . . . to isolate the cells by density gradient centrifugation and fix the cells with paraformaldehyde [sic] before using the cells in a method of characterizing single cells comprising [sic] the concurrent detection of multiple fluorescent probes. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Freshney [sic] on a standard protocols of isolating cells by density centrifugation and the teachings of Ausubel et al. on a standard protocol of fixing cells before contacting said cells with a labeled probe.

Office Action at page 7, lines 11-21.

Applicants respectfully disagree. In order to establish a *prima facie* case of obviousness there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to

modify the reference or to combine the reference teachings. See *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). With respect to the present claims, a *prima facie* case of obviousness has not been established because there is no suggestion, either in Gross *et al.* or in Freshney or Ausubel *et al.* to modify the teaching of Gross *et al.* to arrive at Applicants' claimed invention. As discussed above, Gross *et al.* disclose a *model* study showing detection of breast cancer cells in seeded into a suspension of isolated peripheral blood mononuclear cells. Gross *et al.* do not teach a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. This deficiency is not cured by Freshney or Ausubel *et al.* It is not enough that Freshney and Ausubel *et al.* teach in general "standard protocols" for isolating cells and fixing them.

Gross *et al.* teach that their method would allow detection of cells down to a frequency of 10^{-7} if 4×10^8 PBMCs are analyzed. According to Gross *et al.*, one would need about 200 ml of blood or 2-20 ml of bone marrow. "Since 200 ml of blood will often be impractical, the limiting factor in achieving lower limits of detection will be sample size and not methodology." Gross *et al.* at page 541, first col., second full paragraph. Thus, Gross *et al.* teach that their method is "impractical." In view of this teaching, one of ordinary skill in the art would be led away from modifying Gross *et al.* to achieve the claimed invention.

Finally, Applicants note that the present invention allows one to characterize single circulating cancer cells from as little as 20 ml or less of blood. See Example 4 on pages 25-27 of the present application and new claims 63-66. Such results are truly unexpected in view of the negative comments by Gross *et al.*

Applicants respectfully request that the Examiner withdraw the rejection to claims 1, 2, 4, 5, 9-13, 16, 17, 20, 21, 28, 29, 33-36, 40, 41, 43-46 and 54 under 35 U.S.C. § 103(a) as being unpatentable over Gross *et al.* in view of Freshney and Ausubel *et al.*

The Examiner has rejected claims 1, 2-5, 7-13, 16, 17, 20, 21, 28, 29, 33-36, 40, 41, 43-46 and 53-57 under 35 U.S.C. § 103(a) as being unpatentable over Gross *et al.*, Freshney and Ausubel *et al.* in further view of Anderson *et al.*, *Cancer Res.* 49:4659-4664 (1989). Applicants respectfully traverse this rejection.

Claims 3, 7 and 8 have been canceled thus rendering moot this portion of the rejection.

According to the Examiner:

Gross suggests (p. 537, second column, lines 5-7), but does not teach, an isolated cell preparation obtained by means of a negative selection process. Anderson teaches a method of eliminating breast cancer cells from bone marrow, resulting in a cellular preparation of bone marrow which was obtained by a negative selection process. It would have been *prima facia* [sic] obvious to one of ordinary skill in the art . . . to create a cell preparation that was isolated from the body by means of a negative selection process. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Anderson et al on the method of obtaining a tumor cell-purged bone marrow sample which was generated by negative selection.

Office Action at page 8, lines 4-13.

Applicants respectfully disagree. As discussed above, Gross *et al.* disclose a *model* study showing detection of breast cancer cells seeded into a suspension of isolated peripheral blood mononuclear cells. Gross *et al.* do not teach a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. In fact, Gross *et al.*

teach away from the present invention by suggesting it would be "impractical." This deficiency is not cured by Freshney, Ausubel *et al.*, or Anderson *et al.* In particular, Anderson *et al.* teach removing rather than isolating breast cancer cells. Thus, Anderson *et al.* also teach away from Applicants' invention. Withdrawal of the rejection to claims 1, 2, 4, 5, 9-13, 16, 17, 20, 21, 28, 29, 33-36, 40, 41, 43-46 and 53-57 under 35 U.S.C. § 103(c) is respectfully requested.

The Examiner has rejected claims 1, 2-5, 7-13, 16, 17, 20, 21, 28, 29, 33-36, 40, 41, 43-46, 54 and 58 under 35 U.S.C. § 103(a) as being unpatentable over Gross *et al.*, Freshney and Ausubel *et al.* in further view of Lebkowski *et al.*, *Transplantation* 53:1011-1019 (1992). Applicants respectfully traverse this rejection.

Claims 3, 7 and 8 have been canceled thus rendering moot this portion of the rejection.

According to the Examiner:

Gross does not teach an isolated cell preparation obtained by means of a positive selection process. Lebkowski et al teach an isolated cell preparation of CD34+ cells obtained from bone marrow mononuclear cells by means of a positive selection for CD34+. It would have been *prima facia* [sic] obvious to one of ordinary skill in the art . . . to characterize a single cells [sic] preparation comprising the concurrent detection of multiple fluorescent probes, wherein the single cell preparation was obtained by means of a positive selection. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Lebkowski et al on the 99.9% depletion of tumor cells in cell populations that have undergone positive selection for CD34+ antigen.

Office Action at page 8, line 24, through page 9, line 5.

Applicants respectfully disagree. As discussed above, Gross *et al.* disclose a *model* study showing detection of breast cancer cells seeded into a suspension of isolated peripheral blood mononuclear cells. Gross *et al.* do not teach a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. In fact, Gross *et al.* teach away from the present invention by suggesting it would be "impractical." This deficiency is not cured by Freshney, Ausubel *et al.*, or Lebkowski *et al.* In particular, Lebkowski *et al.* teach removing rather than isolating cancer cells. Thus, Lebkowski *et al.* also teach away from Applicants' invention. Withdrawal of the rejection to claims 1, 2, 4, 5, 9-13, 16, 17, 20, 21, 28, 29, 33-36, 40, 41, 43-46, 54 and 58 is respectfully requested.

The Examiner has rejected claims 1, 3, 5-7, 11, 12, 13-19, 22, 23, 28-33, 37, 38, 40-42, 45, 47, 49, 50 and 54 under 35 U.S.C. § 103(a) as being unpatentable over Fiche *et al.*, *Intl. J. Cancer* 84:511-515 (1999) in view of Ried *et al.* Applicants respectfully traverse this rejection.

Claims 3, 7 and 42 have been canceled thus rendering moot this portion of the rejection.

According to the Examiner:

Fiche et al do not teach a FISH assay using concurrent measurement of fluorescent probes which would hybridize to c-erbB2, the estrogen receptor gene and the progesterone receptor gene. Ried et al teach a method of characterizing chromosomes *in situ* comprising the concurrent detection of up to seven different DNA probes (see discussion in paragraph 8, supra). It would have been *prima facia* [sic] obvious to one of ordinary skill in the art . . . to use a FISH assay to detect c-erbB2 gene, estrogen receptor gene and the progesterone receptor gene concurrently in a single assay. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of

success by the teaching of Ried on the availability of DNA probes labeled with fluorescein isothiocyanate, rhodamine and Cascade Blue that can be visualized simultaneously without spectral overlap.

Office Action at page 9, lines 17-26.

Applicants respectfully disagree. Applicants first note that Fiche *et al.* was published in 1999, which is after Applicants' priority application filed October 29, 1998. Thus, Fiche *et al.* is not prior art to the claims which have priority back to October 29, 1998.

In addition, Fiche *et al.* disclose fluorescence imaging of tumor sections. Fiche *et al.* do not teach or suggest a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. The deficiencies of Fiche *et al.* are not cured by Ried *et al.* who teach the visualization of human metaphase chromosomes with labeled DNA probes. Ried *et al.* do not teach a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. Therefore, the cited references do not teach or suggest the claimed invention.

Withdrawal of the rejection to claims 1, 5-6, 11, 12, 13-19, 22, 23, 28-33, 37, 38, 40-41, 45, 47, 49, 50 and 54 is respectfully requested.

The Examiner has rejected claims 1, 3, 5-7, 11, 12, 13-19, 22, 23, 28-33, 37, 38, 40-42, 45, 47, 48 and 54 under 35 U.S.C. § 103(a) as being unpatentable over Nupponen *et al.*, *Am. J. Pathol.* 153:141-148 (1998), in view of Ried *et al.* Applicants respectfully traverse this rejection.

Claims 3, 6, 7 and 42 have been canceled thus rendering moot this portion of the rejection.

According to the Examiner:

Nupponen et al teach the detection of increased copy number of the c-myc gene and androgen receptor gene as determined in two separate FISH assays on prostate carcinoma cells. Nupponen et al do not teach the concurrent measurement of the c-myc gene and the androgen receptor gene copy number by FISH. Ried et al teach a method of characterizing chromosomes *in situ* comprising the concurrent detection of up to seven different DNA probes (see discussion in paragraph 8, *supra*). It would have been *prima facia* [sic] obvious to one of ordinary skill . . . to use a FISH to detect and measure the copy number of both the c-myc gene and the androgen receptor gene concurrently in a single assay. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Ried on the convenience of using multiple fluorescent probes simultaneously when screening clinical specimens, allowing for a more definitive assessment of gene dosage with less statistical analysis.

Office Action at page 10, lines 10-20.

Applicants respectfully disagree. Nupponen *et al.* disclose fluorescence imaging of tumor sections. Nupponen *et al.* do not teach or suggest a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. The deficiencies of Nupponen *et al.* are not cured by Ried *et al.* who teach the visualization of human metaphase chromosomes with labeled DNA probes. Ried *et al.* do not teach a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. Therefore, the cited references do not teach or suggest the claimed invention.

Withdrawal of the rejection to claims 1, 5, 11, 12, 13-19, 22, 23, 28-33, 37, 38, 40-41, 45, 47, 48 and 54 is respectfully requested.

The Examiner has rejected claims 1, 3, 5-7, 11, 12, 13-19, 22, 23, 28-33, 37-39, 40-42, 45, 47, 48 and 54 under 35 U.S.C. § 103(c) as being unpatentable over

Nupponen *et al.* and Ried *et al.* in further view "of what is well known in the art as exemplified by either Berenson (J of Clinical Investigation, 1995, Vol. 95, pp. 964-972) or Pajor and Bauman (Histochemistry, 1991, vol. 96, pp. 73-81)." Office Action at page 10, lines 24-26. Applicants respectfully traverse this rejection.

Claims 3, 6, 7 and 42 have been canceled thus rendering moot this portion of the rejection.

According to the Examiner:

Nupponen et al. and Ried et al do not teach a method of characterizing single cells comprising the concurrent measurement of multiple fluorescent probes, said fluorescent probes comprising RNA. It is well know [sic] in the art that probes can be either DNA or RNA (Maniatis, 2nd edition, pp. 10.13-10.17 and 10.27-10.37). Either Berenson or Pajor and Bauman teach the use of RNA probes in FISH assays. It would have been *prima facia* [sic] obvious to one of ordinary skill in the art . . . to use an RNA probe in a FISH assay. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Berenson or Pajor and Bauman on the convenience and reliability of obtaining biotinylated RNA probes for use in the FISH assay.

Office Action at page 11, lines 3-11.

Applicants respectfully disagree. Nupponen *et al.* disclose fluorescence imaging of tumor sections. Nupponen *et al.* do not teach or suggest a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. The deficiencies of Nupponen *et al.* are not cured by Ried *et al.* who teach the visualization of human metaphase chromosomes with labeled DNA probes. Ried *et al.* do not teach a method of characterizing single circulating epithelial cancer cells obtained from a body fluid. In addition, neither Berenson nor Pajor and Bauman teach or suggest a method of

characterizing single circulating epithelial cancer cells obtained from a body fluid.

Therefore, the cited references do not teach or suggest the claimed invention.

Withdrawal of the rejection to claims 1, 5, 11, 12, 13-19, 22, 23, 28-33, 37-39, 40-41, 45, 47, 48 and 54 is respectfully requested.

Other Matters

On April 27, 2000, Applicants filed an Information Disclosure Statement, form PTO-1449 (11 sheets), and 35 references. On August 21, 2000, Applicants filed a First Supplemental Information Disclosure Statement, form PTO-1449 (5 sheets), and 15 references. On January 17, 2001, Applicants filed a Second Supplemental Information Disclosure Statement, form PTO-1449 (1 sheet), and 2 references. On January 26, 2001, Applicants filed a Third Supplemental Information Disclosure Statement, form PTO-1449 (1 sheet), and 2 references. Attached herewith are Applicants' postcard receipts date-stamped April 27, 2000, August 21, 2000, January 11, 2001, and January 26, 2001. None of the forms PTO-1449 were initialed and returned by the Examiner with the present Office Action. Applicants respectfully request that the Examiner consider the cited documents and make them of record in the prosecution of the above-identified application by initialing and returning a copy of the forms PTO-1449. If the Information Disclosure Statements have been lost, Applicants respectfully request that the Examiner contact Applicants' undersigned representative who will have copies of the documents delivered to the Examiner.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

At page 1 just after the title, the following paragraph has been added.

This claims priority to U.S. Provisional Application 60/106,118, filed October 29, 1998.

The paragraph bridging pages 8 and 9 of the present application has been canceled and the following paragraph has been inserted in place thereof:

In one embodiment of the invention, a characterization protocol may include combination staining (e.g., fluorescence staining) and fluorescent in situ hybridization (FISH) (FISH protocol and probes may be found, for example, in Meyne et al., in Methods of Molecular Biology, 33:63-74 (1994)). For example, specific nucleic acid sequences are suitable as probes for cancer cells. In particular, molecular probe design may include, but is not limited to, chromosomal centromere probes such as those for Chromosome 18, 5'-Cy3-TT-Cy3-TT-Cy3-GAG ATG TGTGTACTCACACTAAG AGAATTGAACCACCGTTT GAAAGGAGC-3' (SEQ ID NO: 1); Chromosome 17, 5'-CY5-TT-CY5-TT-CY5-TGTTTC AAA CGT GAA CTT TGA AAG GAA AGT TCA ACT CGG GGA TTT GAA TG-3' (SEQ ID NO: 2); Chromosome 7, 5'-CY5-TT-CY5-TT-CY5-GCT GTG GCA TTT TCA GGT GGA GAT TTC AAG CGA TTT GAG GAC AAT TGC AG-3' (SEQ ID NO:3); and mRNA Probe Design such as Cytokeratin 14 mRNA probe, 5'-CY3-TT-CY3-TT-CY3-GGA TTT GGC GGC TGG AGG AGG TCA CAT CTC TGG ATG ACT GCG ATC CAG AG-3' (SEQ ID NO:4); Cytokeratin 19 mRNA [Probe] probe, 5'-CY3-TT-CY3-TT-CY3-ATC TTG GCG AGA TCG GTG CCC GGA GCG GAA TCC ACC TCC ACA CTG ACC TG-3' (SEQ ID NO:5); MUC I (EPISIALIN) mRNA Probe, 5'-FITC-TT-FITC-TT-FITC-TTG AACTGTGTCTCCACGTCGTGGAC ATTGA TGGT AC C TTCTCGG AAG GC-3' (SEQ

ID NO: 6); and Estrogen-mRNA probe, 5'-CY5-TT-CY5-TT-CY5-GTG CAG ACC GTG TCC CCG CAG GGC AGA AGG CTG CTC AGA AAC CGG CGG GCC AC-3' (SEQ ID NO: 7); and in [particularly] particular, probes for the centromere regions of chromosome 7 (e.g., CGATTGAGGACAATTGCAG (SEQ ID NO: 8)), chromosome 18 (e.g., GTACTCACAC TAAGAGAATT GAACCACCGT (SEQ ID NO:9)), chromosome X (e.g., GACGATGGAGTTAACTCAGG (SEQ ID NO:10), TCGTTGGAACGGG AATAA T T C C C A T A A C T A A A C A C A A A C A (SEQ ID NO:11), AAGCCTTCCCTTATCTCACAGAAAGA (SEQ ID NO:12)) may be targeted. A sequence length of about 20 to about 60 nucleotides can be used, preferably a length of about 40-45. Cancer cells can also be identified by polymerase chain reaction (PCR) techniques, which techniques and probes are well known to those in the art.

Claims 3, 6, 7, 8, 26, 27, 42 and 57 have been canceled.

New claims 59-70 have been added.

The claims have been amended as follows:

1. (Once amended) A method of characterizing single circulating epithelial cancer cells obtained from a body fluid comprising the concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in [a] said single cell using [fluorescent] fluorescence microscopy.

34. (Once amended) The method of claim 1, wherein said probe [comprises a biological probe] is directed to a cellular target and is not a nucleic acid.

35. (Once amended) The method of claim 34, wherein said [biological] probe comprises a protein or peptide.

36. (Once amended) The method of claim 35, wherein said [protein] probe is an antibody.

37. (Once amended) The method of claim 1, wherein said probe [comprises a molecular probe] is a nucleic acid directed to a cellular target.

38. (Once amended) The method of claim 37, wherein said [molecular] probe comprises DNA [or a DNA sequence thereof].

39. (Once amended) The method of claim [38] 37, wherein said [molecular] probe comprises RNA [or an RNA sequence thereof].

40. (Once amended) The method of claim 1, wherein said probes comprise
(i) [biological] probes which are directed to a cellular target and are not a nucleic acid,
(ii) [molecular] probes which are a nucleic acid directed to a cellular target, or
(iii) a combination of (i) and (ii) [biological and molecular probes].

41. (Once amended) The method of claim 40, wherein said [biological] probes are selected from the group consisting of identification probes, proliferation probes, cell cycle arrest probes, oncogenes, [viral, bacterial] and hormonal probes.

43. (Once amended) The method of claim 40, wherein said probes comprises an epithelial cell-specific [probes] probe.

44. (Once amended) The method of claim 40, wherein the probes comprise a tissue-specific [probes] probe.

47. (Once amended) The method of claim 40, wherein said [biological and molecular] probes are used to detect a hormone receptor or a hormone receptor gene for the enumeration of copy number.

53. (Once amended) A method of characterizing a single circulating epithelial cancer cell preparation obtained from a body fluid comprising adhering a circulating epithelial cancer cell preparation to be characterized onto a surface, fixing said cell preparation with a fixative solution, incubating [such a] said cell surface containing fixed cells with multiple probes directed to desired cellular markers, wherein said multiple probes have the ability to fluoresce when excited at different wavelengths, and examining the cells by [fluorescent] fluorescence microscopy for identification of positive cells for each selected cellular marker, wherein said cancer cell preparation is isolated from a body fluid using a negative selection process.

54. (Once amended) A method of establishing a characterization profile of a circulating epithelial cancer cell obtained from a body fluid comprising [a method of] characterizing a single cell environment, wherein the concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in [a] the single cell using [fluorescent] fluorescence microscopy.

In the Sequence Listing:

A Sequence Listing has been added after the claims in the application.